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A new *Hibiscus*-feeding species of *Bucculatrix* (Bucculatricidae) from the Ogasawara Islands, Japan

Sadahisa Yagi[1,2], Johei Oku[1], Toshiya Hirowatari[1,2]

1 *Entomological Laboratory, Faculty of Agriculture, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan; E-mail: yagi.sadahisa@gmail.com, z610034b@yahoo.co.jp, hirowat_t@agr.kyushu-u.ac.jp*

2 *Insect Science and Creative Entomology Center, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan*

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Abstract. This study describes *Bucculatrix flavimaculata* **sp. nov.**, a new species of *Bucculatrix* from the Ogasawara Islands in Japan, feeding on *Hibiscus*. Photographs of adult male and female genitalia and their larval biology (e.g., host plants and feeding habits) are shown. We constructed a phylogenetic tree based on cytochrome c oxidase subunit I (COI-5P) DNA barcode sequences, including those of some Japanese *Bucculatrix* species related to *B. flavimaculata* **sp. nov**. In addition, we discuss the relationships within the species group.

Introduction

The genus *Bucculatrix* comprises mostly leaf-mining moths, with approximately 300 species known globally (Davis and Robinson 1998; van Nieukerken et al. 2011), with several recently described species (e.g. Tokár and Laštůvka 2018; Liao et al. 2019). Braun (1963) revised the genus *Bucculatrix* for the Nearctic region and divided it into eight sections based on their genitalia. These sections were mainly correlated with the host plant. Seksjaeva (1994, 1998) revised the Palearctic species into 11 species groups. Similarly, Baryshnikova (2008) revised the Holarctic species and divided them into 16 species groups using phylogenetic analyses based on morphological and biological (e.g., the larval life history) characteristics. Subsequently, Kobayashi et al. (2010) revised the 23 Japanese *Bucculatrix* species and presented 10 species groups distributed in Japan based on their morphological characteristics.

Several Malvaceae-feeding *Bucculatrix* species are known from the Nearctic and Palearctic regions. Most of these species belong to one species group (viz. Section VIII sensu Braun, 1963; the *lavaterella* group sensu Baryshnikova, 2008) based on the similarity of the male and female genitalia (Braun 1963; Seksjaeva 1994; Baryshnikova 2007). In contrast, two Malvaceae-feeding species recorded from Japan, *B. firmianella* Kuroko, 1982 and *B. hamaboella* Kobayashi, Hirowatari & Kuroko, 2009, were considered to belong to another species group (viz. Section I sensu Braun, 1963; the *magnella* group sensu Baryshnikova, 2008) based on the similarity of male and female genitalia and the stem-boring feeding habit (Kobayashi et al. 2009).

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The first and second instar larvae of *Bucculatrix* are leaf miners that form short, very narrow, tortuous, or spiral mines. The third and fourth instars feed usually externally on the surface of the leaf and usually leave the "skeletonized" feeding trace and spin a cocoon-shaped web "cocoonet" for moulting (Braun 1963; Kuroko 1964; Kobayashi et al. 2010).

Some studies on *Bucculatrix* have used DNA barcode sequences (partial cytochrome c oxidase subunit I, COI-5P) for species identification (van Nieukerken et al. 2012; Vargas-Ortiz et al. 2018; Liao et al. 2019). However, most of these data are limited to Europe and the USA, and only a few species in Asia are registered in the GenBank or BOLD databases.

In the Ogasawara Islands, the endemic Malvaceae plant *Hibiscus glaber* (Matsum. ex Hatt.) Matsum. ex Nakai is widely distributed, and one unknown species belonging to Gracillariidae is known to feed on this plant (Takeuchi and Ohbayashi 2006). During a survey of the Ogasawara Islands, we discovered not only gracillariid mines but also unknown small mines and "skeletonized" feeding traces. In the course of the survey, we found that these latter traces are made by an unknown species of the genus *Bucculatrix*. We conclude that this is a new species based on morphology and DNA barcoding analyses. We here describe the adult morphology of this species, including the male and female genitalia. This study also provides some aspects of larval biology and a preliminary phylogenetic tree of the genus *Bucculatrix* based on the partial COI sequences, with a brief discussion on the phylogenetic relationships within the group.

Materials and methods

Materials and observations

All specimens were collected by rearing larvae from the host plant or sweeping them from Chichijima and Hahajima, both in the Ogasawara Islands. All samples were deposited at the Entomological Laboratory of Kyushu University (ELKU). The morphological characteristics were observed using a Leica S9D stereoscopic microscope. The genitalia were dissected, stained with Chlorazol black E and mounted in Euparal. Morphological terminology follows that of Kobayashi et al. (2010) and Liao et al. (2019). The scientific names of plants follow Yonekura and Kajita (2003). A distribution map was created with QGIS 3.10.11 (QGIS 2020) using data from Global Map Japan (Global Map Japan version 2.2 Vector data, https://www.gsi.go.jp/kankyochiri/gm_japan_e.html).

DNA analysis

The specimens collected by the first author were used for DNA analysis (Table 1). Total DNA was extracted from the abdomen using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We amplified the fragment of the partial mitochondrial COI gene using the primer sets LCO1490/HCO2198 (Folmer et al. 1994) or LepF1/LepR1 (Hebert et al. 2003) (Table 1) and the KOD One® PCR Master Mix -Blue- (TOYOBO CO., LTD., Osaka, Japan). Amplification conditions followed the manufacturer's protocol, except for the annealing temperature, which was either 50 °C or 53 °C. The amplicons were purified using ExoSAP-IT Express (Thermo Fisher Scientific Inc., Massachusetts, USA). The cycle sequences reactions and bidirectional sequencing were conducted using premixed Sanger sequencing services (Azenta Japan Corp., Tokyo, Japan).

Table 1. The list of Japanese *Bucculatrix* used for DNA analysis.

Species name	Sample ID	sex	location	collecting date	Genbank accession number	BIN	primer sets for PCR
Bucculatrix flavimaculata sp. nov. (holotype)	SaY892	male	Ogasawara-mura, Chichijima Is., Higashidaira	18. vi. 2022	OR076329	BOLD:AFB6292	LCO1490/ HCO2198
Bucculatrix flavimaculata sp. nov. (paratype)	Jo000031	male	Ogasawara-mura, Chichijima Is., Higashidaira	26. vi. 2022	OR076331	BOLD:AFB6292	LepF1/ LepR1
Bucculatrix flavimaculata sp. nov. (paratype)	Jo000030	male	Ogasawara-mura, Hahajima Is., Chibusayama	21. vi. 2022	OR076326	BOLD:AFB5723	LepF1/ LepR1
Bucculatrix flavimaculata sp. nov. (paratype)	Jo000032	male	Ogasawara-mura, Hahajima Is,, Igumadani	22. vi. 2022	OR076318	BOLD:AFB5723	LepF1/ LepR1
Bucculatrix flavimaculata sp. nov. (paratype)	Jo000033	male	Ogasawara-mura, Hahajima Is., Igumadani	22. vi. 2022	OR076330	BOLD:AFB5723	LepF1/ LepR1
Bucculatrix flavimaculata sp. nov. (paratype)	SaY737	male	Ogasawara-mura, Hahajima Is., Igumadani	22. vi. 2022	OR076320	BOLD:AFB5723	LCO1490/ HCO2198
Bucculatrix flavimaculata sp. nov. (paratype)	SaY887	female	Ogasawara-mura, Hahajima Is., Igumadani	22. vi. 2022	OR076323	BOLD:AFB5723	LCO1490/ HCO2198
Bucculatrix flavimaculata sp. nov. (paratype)	SaY888	female	Ogasawara-mura, Hahajima Is., Igumadani	22. vi. 2022	OR076324	BOLD:AFB5723	LCO1490/ HCO2198
Bucculatrix splendida	SaY878	male	Hokkaido, Futami-gun, Yakumo-cho, Unseki-toge Pass	8. x. 2021	OR076321	BOLD:AFC1518	LCO1490/ HCO2198
Bucculatrix citima	SaY893	female	Futami-gun, Yakumo-cho, Unseki-toge Pass	15. ix. 2017	OR076325	BOLD:ACB4134	LCO1490/ HCO2198
Bucculatrix notella	SaY896	male	Chino-shi , Tamagawa	18. vii. 2018	OR076327	BOLD:ADL9412	LCO1490/ HCO2198
Bucculatrix firmianella	SaY897	male	Ishikari-shi, Oyafune-cho	8. vi. 2018	OR076316	BOLD:AFD6196	LCO1490/ HCO2198
Bucculatrix thoracella	SaY898	male	Fukuoka-shi, Higashi-ku, Hakozaki	19. v. 2021	OR076317	BOLD:AFD3759	LCO1490/ HCO2198
Bucculatrix muraseae	SaY899	female	Fukuoka-shi, Sawara-ku, Itaya, Mt. Sefuri	29. vii. 2019	OR076315	BOLD:AFC2937	LCO1490/ HCO2198
Bucculatrix damaryella	SaY900	female	Takarazuka-shi, Sakaino, Yaganitani	24. ix. 2017	OR076322	BOLD:AFD6197	LCO1490/ HCO2198
Bucculatrix hamaboella	SaY901	male	Senboku-shi, Nishiki-cho, Saimyoji, Katagiri	24. vi. 2021	OR076328	BOLD:AFC1863	LCO1490/ HCO2198
Bucculatrix serratella	SaY912	female	Itoshima-shi, Maebaru, Izumi-gawa	18. vii. 2020	OR076319	BOLD:AFD4055	LCO1490/ HCO2198

The sequences obtained were manually aligned using MEGA 7.0.26 (Kumar et al. 2016). Then intraspecific uncorrected pairwise distances (p-distances) were calculated using MEGA 7.0.26 (Kumar et al. 2016). For visualization, a Neighbor Joining tree was constructed under the uncorrected pairwise distances method using MEGA 7.0.26 (Kumar et al. 2016). The sequence data were compared with the sequences registered in BOLD Systems, and similar species were searched using the BOLD identification engine (https://www.boldsystems.org/index.php/IDS_OpenIdEngine). To construct a preliminary phylogenetic tree, we acquired sequences from BOLD, including all described *Bucculatrix* species with registered COI barcode sequences longer than 500 base pairs and two sequences of unidentified specimens that were similar to the new species (Suppl. material 1). The sequence of *Ogmograptis scribula* Meyrick, 1935 (Bucculatricidae) was used as an outgroup. Owing to the heterogeneity of the third codon positions of the COI barcode region, this partition was binarized (RY coding). A phylogenetic tree based on maximum likelihood (ML) estimation was constructed using the IQ-TREE web server, version 1.6.12 (Trifinopoulos et al. 2016) with an auto-substitution model selection, ultrafast bootstrap analysis with 1000 repetitions, and Shimodaira-Hasegawa-like approximate likelihood ratio tests (SH-aLRT) with 1000 repetitions. The treefile was visualized using Figtree v1.4.4 (Rambaut 2018) to prepare the figure.

Results

Taxonomy

Bucculatrix flavimaculata Yagi & Hirowatari, sp. nov.

https://zoobank.org/ DFA961A1-9015-4911-A3A3-EC0C93C35BF8

Japanese name: munin-kimadara-chibiga

Diagnosis. This species can be easily distinguished from other *Bucculatrix* species distributed in the surrounding biogeographic regions by its fuscous forewings mixed with four yellowish cream marking at or near costal margin. The male genitalia are similar to those of *B. firmianella* (Group 1 sensu Kobayashi et al. 2010) in that they share a long and slender socius, a broad valva, and an elongated vinculum. Moreover, they also share an elongated ductus bursae in the female genitalia. However, this species can be distinguished by the trapezoid valva (triangular in *B. firmianella*) and small-scale sac (large in *B. firmianella*) in males and the broad lamella antevaginalis with some wrinkles in females (without wrinkles in *B. firmianella*).

The male and female genitalia are also similar to those of *Bucculatrix serratella* Kobayashi et al., 2010 (Groups 8 sensu Kobayashi et al. 2010), which share a long and slender socius and rounded apex of the valva in the male genitalia. They also share the lamella antevaginalis with wrinkles in the female genitalia. However, the species can be distinguished by the absence of a juxta in the male genitalia and long ductus bursae (short in *B. serratella*) in the female genitalia.

Description. Adult (Figs 1, 2). No sexual dimorphism. Wingspan 3.8–6.6 mm (n = 16; holotype: 6.6 mm). Forewing length 1.7–3.0 mm (n = 16, holotype 3.0 mm).

***Head*:** Frons pale dark brown with cream scales, apically cream. Vertex cream with dark brown hair-like scales. Antennae filiform, 4/5 of forewing length; scape cream, forming eye-caps with cream hair-like scales ventrally; pedicel cream with dark greyish-brown scales; flagellum dark greyish-brown. Proboscis yellowish cream without scales. Labial palpus short, cream.

***Thorax*:** Fuscous with cream scales dorsally, dark greyish-brown laterally. Forecoxa to forefemur cream; fore tibia greyish-brown dorsally, yellowish-cream ventrally; fore tarsomere yellowish-cream basally and ventrally, greyish-brown dorso-apically; mid-coxa to mid-femur cream; mid-tibia greyish-brown with yellowish cream marking middle dorsally; mid-tarsomere yellowish cream basally and ventrally, greyish-brown dorso-apically; hind coxa to hind femur cream. hind tibia greyish-brown with long hairs dorsally, yellowish cream with long hairs ventrally; hind-tarsomere yellowish cream basally and ventrally, greyish-brown dorso-apically. Forewing lanceolate, ground colour fuscous with four inconspicuous cream to dark yellow spots or markings on basally, 1/4, 1/2, 3/4 of forewing; basal marking slender strip, not reaching inner margin; marking at 1/4 divided into two or three spots at middle of forewing; spot at 1/2 at costal margin; marking at 3/4 divided into two spots at costal margin and tornus, tornus spot larger than costal spot; black erected spot at basal 2/5 of forewing near inner margin (Figs 23, 24); fringe dark greyish-brown. Hindwing greyish-brown; fringe greyish-brown.

***Abdomen*:** Greyish-brown dorsally, cream ventrally. Scale sac small, ovate.

Male genitalia (Figs 3–9): Uncus and gnathos absent. Socius elongated, apical half with setae, apex slightly broad. Tegumen narrow with one pair of small lobes with setae laterally. Vinculum developed and elongated with a broadly roundish tip. Valva trapezoid, apex slightly broad and roundish. Anellus membranous covered with minute sclerites (Fig. 8). Phallus elongated, J-shaped, tapered toward tip, slightly curved and crinkled posteriorly; with a U-shaped lobe basally.

Figures 1, 2. Adults of *Bucculatrix flavimaculata* sp. nov. from the Ogasawara Islands. **1.** Male, holotype; **2.** Female, paratype, genitalia slide no. SY1446. Scale bars: 3.0 mm.

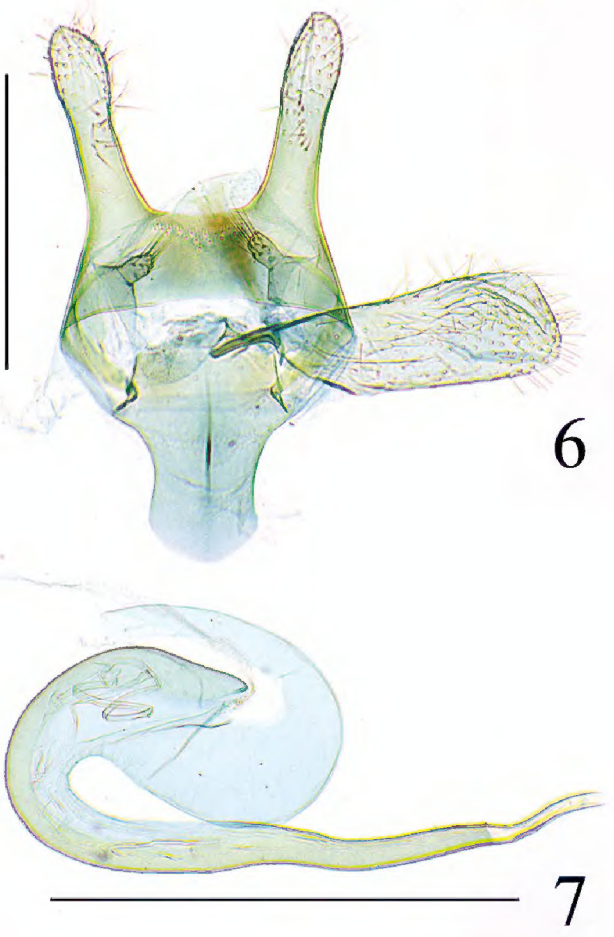
Female genitalia (Figs 10–12). Papilla analis moderately long, tapering toward the tip. Apophysis posterioris slender, longer than eighth segment. Apophysis anterioris absent. Ostium bursae broad. Lamella antevaginalis broad with several horizontal wrinkles. Antrum elongated, bar-shaped, and slightly shorter than posterior apophysis. Ductus bursae narrow and elongated, longer than corpus bursae. Corpus bursae anteriorly broad, posteriorly tapering toward ductus bursae, with significant signa. Signa arranged in two rows; each signum forming posterior row with sharp internal spine; that of anterior row with blunt internal process. Ductus seminalis slender, arising near border of ductus bursae and corpus bursae.

Type material. Holotype. ♂; '[JPN: Ogasawara Isls.] Higashidaira, Chichijima Is., Ogasawara-mura; 18.vi.2022 larva; S. Yagi leg.', 'M2808; Host: *Hibiscus glaber*; 4.vii.2022 em.', genitalia slide no. SY1459(♂); Sample ID for DNA analysis: SaY892; Collection ID: ELKU-I-L-Bonin 0000155; deposited in ELKU.

Paratypes. [Chichijima Is.] • 1♂; same locality and collector; 26.vi.2022 Sweeping; genitalia slide no./Sample ID: SY1457(♂)/Jo000031; ELKU. [Hahajima Is.] • 6♂7♀; Ogasawara-mura, Hahajima Is., Igumadani; 22.vi.2022; Host: *Hibiscus glaber*; 2–6.vii.2022 em.; S. Yagi leg.; genitalia slide no./Sample ID: SY1307(♂)/SaY737, SY1446(♀)/SaY887, SY1447(♀)/SaY888, Bonin000032(♂)/Jo000032, Bonin000033(♂)/Jo000033; ELKU • 1♂1♀; Ogasawara-mura, Hahajima Is., Chibusayama; 21.vi.2022 larva; Host: *Hibiscus glaber*; 2–4.vii.2022 em.; S. Yagi leg.; genitalia slide no./Sample ID: SY1458/Jo000030; ELKU.

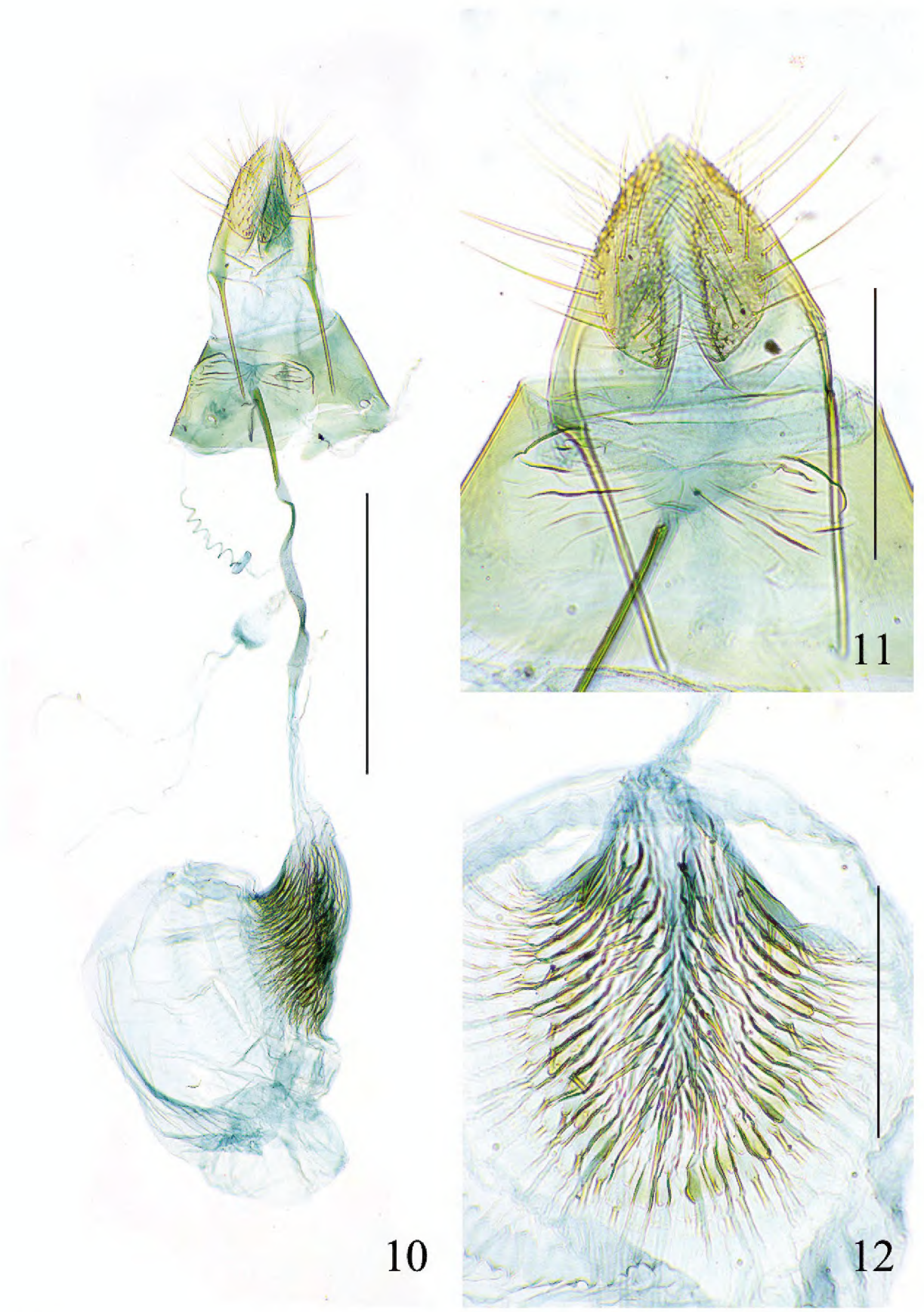
Host plant. *Hibiscus glaber* (Matsum. ex Hatt.) Matsum. ex Nakai (Malvaceae).

Biology (Figs 13–24). Eggs were mainly deposited on the upper side (n = 10) and sometimes on the underside of the leaf (n = 2), near a leaf vein or leaf edge. The young larvae are leaf miners. Leaf mining usually starts as a serpentine mine and changes to a simple linear mine. Subsequently, the larvae exit the mine, feed on the upperside or underside of the leaf, and leave the typical *Bucculatrix* "skeletonized" feeding trace after only the epidermis is consumed and veins remain. The cocoonet is typically located on the underside of the leaf. All larvae of the discovered species are pale green, except for the last instar. The last instar larva has dorsal fuscous and cream horizontal stripes and two lateral white markings on each segment. The spindle-ribbed cocoon is pale cream at first, and then cream. Adults and larvae were observed at almost the same time from mid-June to late June. Our collections demonstrate that this species may be multivoltine.



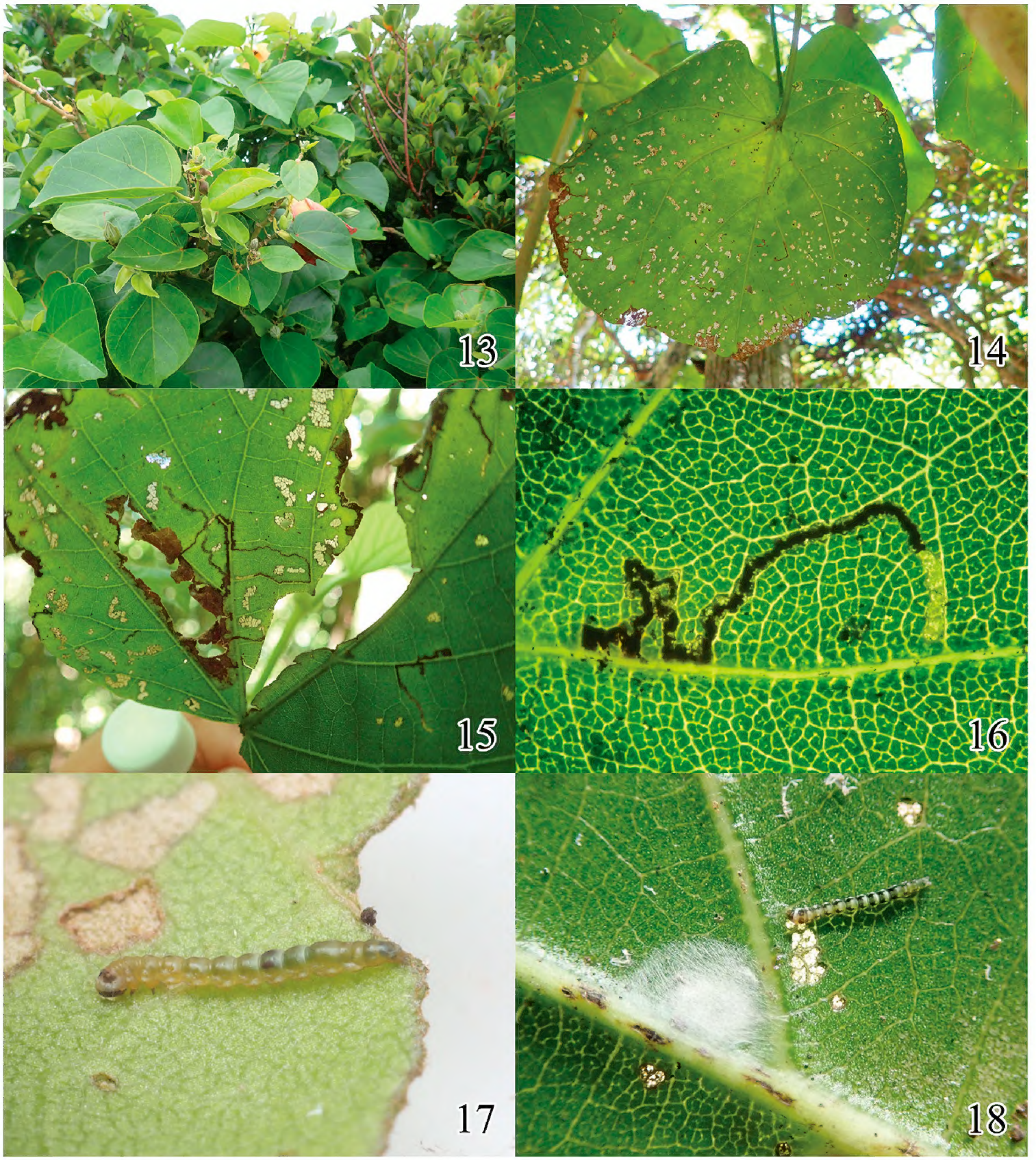
Figures 3–9. Male genitalia of *Bucculatrix flavimaculata* sp. nov. **3.** Holotype, genitalia slide no. SY1459; **4, 5, 8, 9.** Paratype, genitalia slide no. Bonin000032; **6, 7.** Paratype, genitalia slide no. SY1457; **3, 4.** Whole genitalia, lateral view; **5.** Whole genitalia, dorsal view; **6.** Genitalia except phallus and left valva, ventral view; **7.** Phallus; **8.** Anellus, ventral view; **9.** Scale sac. Scale bars: 300 μm (**3, 4, 5, 6, 7, 9**); 100 μm (**8**).

Distribution (Fig. 25)**.** Japan: Ogasawara Islands (Chichijima, Anijima, Hahajima, and Mukohjima Islands). Adults were collected from Chichijima and Hahajima Islands. However, many feeding traces on the same host that we presume are from the newly described species were observed on Anijima and Mukohjima Islands (Figs 14, 25).



Figures 10–12. Female genitalia of *Bucculatrix flavimaculata* sp. nov., paratype. **10.** Whole genitalia, ventral view, genitalia slide no. SY1446; **11.** Terminal segment, genitalia slide no. SY1447; **12.** Signum, genitalia slide no. SY1447. Scale bars: 500 μm (**10**); 200 μm (**11, 12**).

DNA data (Figs 26, 27, Suppl. material 2). The DNA barcode sequences are deposited into the GenBank database under the accession numbers listed in Table 1 and uploaded to the BOLD system in the public dataset DS-BUCBONIN. The intraspecific p-distances of *B. flavimaculata* sp. nov. are 0–2.74% (n = 6) (Suppl. material 2). The largest p-distances were found between two specimens collected from Chichijima Island (BIN, BOLD:AFB6292) compared to six Hahajima Island specimens (BIN, BOLD:AFB5723) (2.43–2.74% pairwise distances) (Fig. 26, Suppl. material 2).



Figures 13–18. Biology and habitat of *Bucculatrix flavimaculata* sp. nov. Biology and habitat of *Bucculatrix flavimaculata* sp. nov. **13.** Host plant, *Hibiscus glaber* at Chibusayama, Hahajima Is.; **14.** Leaf with mines and traces of feeding at Mukohjima Is.; **15.** Leaf with mines and traces of feeding at Igumadani, Hahajima Is.; **16.** Mine; **17.** Larva; **18.** Last instar larva with cocoonet.

The DNA barcode of *B. flavimaculata* sp. nov. (Sample ID: SaY892) is closest to that of a Bucculatricidae species collected in Selangor, the peninsula of Malaysia (BIN, BOLD:ACR3285, Sample ID: BIOUG17088-B08, Process ID: GMMGT2203-14), based on the BOLD identification engine, with 6.46% pairwise divergence.

According to the phylogenetic tree constructed based on the COI barcode region, *Bucculatrix flavimaculata* is closely related to *B. firmianella*, although the ultra-fast bootstrap support and

Figures 19–24. Biology and habitat of *Bucculatrix flavimaculata* sp. nov. (continued) **19, 20.** Last instar larva; **21.** Cocoon; **22.** Cocoon with pupal exuviae; **23, 24.** Adult.

SH-aLRT support values are low or unsupported (86% and 24.2% respectively) (Fig. 27). Moreover, *B. flavimaculata* and *B. firmianella* form a clade with two unidentified species from Malaysia, *B. hamaboella*, *B. regaella* Chrétien, 1907 (Malvaceae feeder), and *B. cordiaella* Davis & Landry, 2002 (Boraginaceae feeder) with high SH-aLRT support (97.1%, Fig. 27).

Etymology. The name of the new species is derived from the Latin flavus, "yellowish" and macula, "spots", referring to the colour of the forewing and the spot markings.

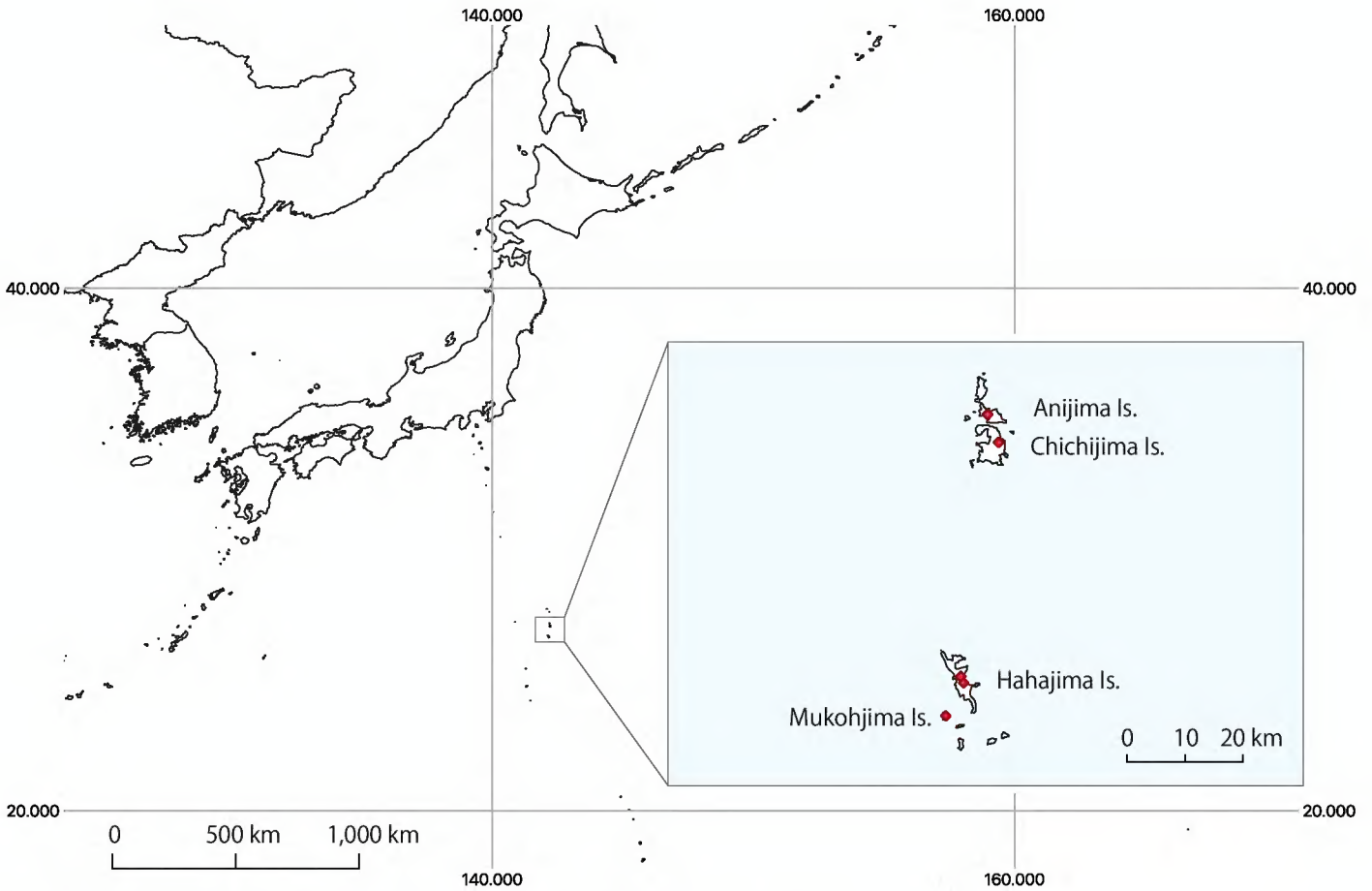


Figure 25. Distribution map of *Bucculatrix flavimaculata* sp. nov.

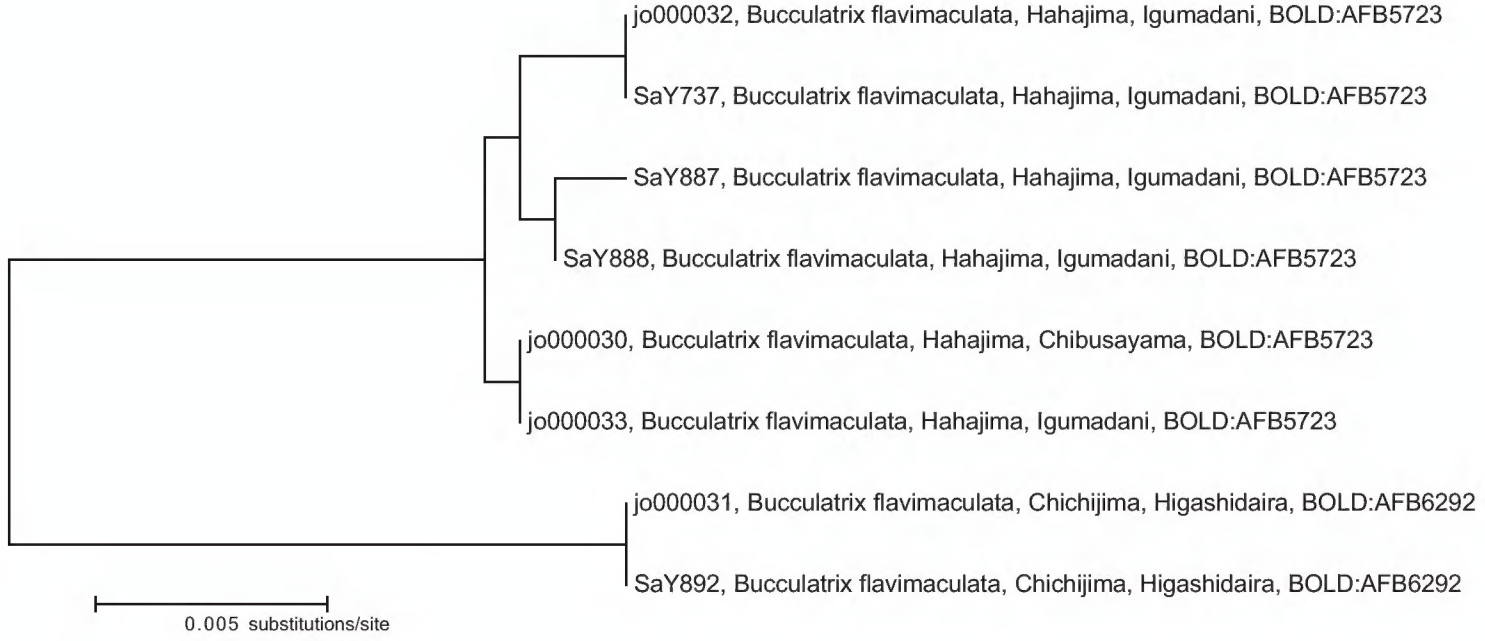

jo000032, Bucculatrix flavimaculata, Hahajima, Igumadani, BOLD:AFB5723
SaY737, Bucculatrix flavimaculata, Hahajima, Igumadani, BOLD:AFB5723
SaY887, Bucculatrix flavimaculata, Hahajima, Igumadani, BOLD:AFB5723
SaY888, Bucculatrix flavimaculata, Hahajima, Igumadani, BOLD:AFB5723
jo000030, Bucculatrix flavimaculata, Hahajima, Chibusayama, BOLD:AFB5723
jo000033, Bucculatrix flavimaculata, Hahajima, Igumadani, BOLD:AFB5723
jo000031, Bucculatrix flavimaculata, Chichijima, Higashidaira, BOLD:AFB6292
SaY892, Bucculatrix flavimaculata, Chichijima, Higashidaira, BOLD:AFB6292
0.005 substitutions/site

Figure 26. Neighbor Joining tree of the genus *Bucculatrix flavimaculata* sp. nov. based on the DNA barcode region. The labels provide data on Sample ID, specific name, island, and exact sites. Branch lengths are proportional to genetic distances and the scale bar indicates 0.005 substitutions/site. Within sample names, species names and BIN IDs are followed by sample IDs.

Remarks. In Japan, *Bucculatrix hamaboella* is known to feed on *Hibiscus hamabo* Siebold et Zucc. (Kobayashi et al. 2009). However, its morphological and molecular characteristics are markedly different from *B. flavimaculata*. Thus, it does not seem to be a closely related species. The young larva of *B. hamaboella* is a leaf miner that forms a long red linear mine. In later instars, the larva becomes a stem borer (Kobayashi et al. 2009).

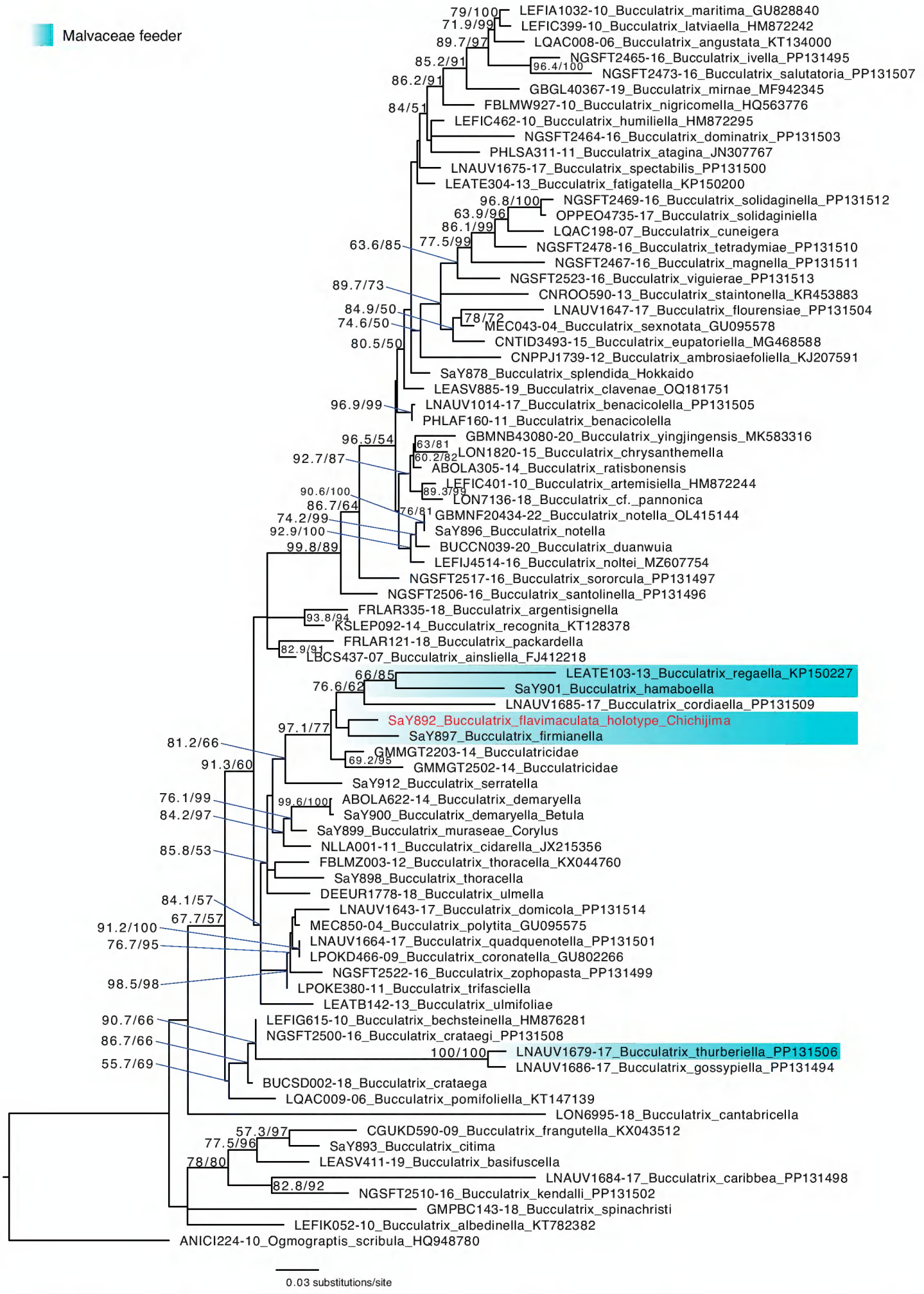


Figure 27. ML tree of the genus *Bucculatrix* based on the DNA barcode region generated by IQ-TREE web server. Numbers near each node are SH-aLRT support (%) / ultrafast bootstrap support (%). Numbers are indicated in case both support values are higher than 50 (%) / 50 (%). Light blue highlights indicate the species known to feed Malvaceae. Branch lengths are proportional to genetic distances and the scale bar indicated 0.03 substitutions/site.

Discussion

Bucculatrix flavimaculata exhibits an intraspecific variation of 2.43–2.74% in DNA barcodes between the populations of the Chichijima and Hahajima Islands (ca. 50 km apart). In Lepidoptera, a p-distance of 2–3% in the DNA barcode region is often considered as a useful threshold to assess cryptic species (Hebert et al. 2003; Huemer and Wiesser 2023). The male genitalia also show some variation in the shape of the valva and phallus. We hypothesize that the morphological variation observed occurs within the populations rather than between the populations of each island, but we cannot exclude that it is an artefact caused by the angle of observation (due to different embedding). However, a detailed morphometric analysis was not performed to test potential morphological differences in the genitalia.

According to Kobayashi et al. (2010), the Japanese Malvaceae feeders *B. firmianella* and *B. hamaboella* belong to Group 1. In addition, they correspond to the *magnella* group sensu Baryshnikova (2008) and Section I sensu Braun (1963). However, the Japanese Malvaceae feeders did not seem to belong to the *magnella* group because they did not form a clade with *B. magnella* Chambers, 1875 in the COI tree. This result is congruent with morphological characters because Japanese Malvaceae feeders lack teeth at the apex of the phallus and short blunt cone setae at the apical area of the valva, which are common characteristics of this group. A more comprehensive re-examination is necessary to determine which species groups sensu Baryshnikova (2008) and Braun (1963) correspond to Group 1 of Kobayashi et al. (2010), as some characteristics are also similar to those of species belonging to other species groups. Other Holarctic Malvaceae feeders belong to the *lavaterella* group sensu Baryshnikova (2008). In the COI tree, *B. thurberiella* Busck, 1914 and *B. gossypiella* Morrill, 1927 are closely related sister species but they do not group closely with *B. flavimaculata* and related taxa, concordant with previous morphological studies. Thus, Malvaceae feeding seems to have arisen independently at least twice within *Bucculatrix*.

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References

Braun AF (1963) The genus *Bucculatrix* in America North of Mexico (Microlepidoptera). Memoirs of the American Entomological Society 18: 1–208.

Baryshnikova SV (2007) Two new Palaearctic species of the genus *Bucculatrix* (Lepidoptera: Bucculatricidae). Zoosystematica Rossica 16: 285–288. https://doi.org/10.31610/zsr/2007.16.2.285

Baryshnikova SV (2008) Systematics and phylogeny of the genus *Bucculatrix* (Lepidoptera: Bucculatricidae) with special attention to larval mode of life and trophic relationships. Trudy Zoologicheskogo Instituta Rossiskoy Akademii Nauk 312 (1–2): 95–113. [In Russian] https://doi.org/10.31610/trudyzin/2008.312.1-2.95

Davis DR, Robinson GS (1998) The Tineoidea and Gracillarioidea. In: Kristensen NP (Ed.) Lepidoptera, moths and butterflies. Evolution, systematics, and biogeography. Handbook of Zoology 4. De Gruyter, Berlin, 91–117. https://doi.org/10.1515/9783110804744.91

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial Cytochrome C Oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Hebert PD, Cywinska A, Ball SL, Dewaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B: Biological Sciences 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Huemer P, Wieser C (2023) DNA Barcode Library of Megadiverse Lepidoptera in an Alpine Nature Park (Italy) Reveals Unexpected Species Diversity. Diversity 15(2): 214. https://doi.org/10.3390/d15020214

Kobayashi S, Hirowatari T, Murase M, Kuroko H (2009) A new stem-borer of the genus *Bucculatrix* (Lepidoptera: Bucculatricidae) from Japan, with description of the life history. Entomological Science 12: 84–90. https://doi.org/10.1111/j.1479-8298.2009.00307.x.

Kobayashi S, Hirowatari T, Kuroko H (2010) A revision of the Japanese species of the family Bucculatricidae (Lepidoptera). Transactions of the Lepidopterological Society of Japan 61(1): 1–57. https://doi.org/10.18984/lepid.61.1_1

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Kuroko H (1964) Revisional studies on the family Lyonetiidae of Japan (Lepidoptera). Esakia 4: 1–61. https://doi.org/10.5109/2349

Liao C, Yagi S, Kobayashi S, Huang G (2019) Two new species of *Bucculatrix* Zeller (Lepidoptera: Bucculatricidae) from China. Zootaxa 4624(3): 322–336. https://doi.org/10.11646/zootaxa.4624.3.2

Nieukerken EJ van, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, Wahlberg N, Yen SH, Zahiri R, Adamski D, Baixeras J, Bartsch D, Bengtsson BÅ, Brown JW, Bucheli SR, Davis DR, De Prins J, De Prins W, Epstein ME, Gentili-Poole P, Gielis C, Hättenschwiler P, Hausmann A, Holloway JD, Kallies A, Karsholt O, Kawahara AY, Koster S, Kozlov MV, Lafontaine JD, Lamas G, Landry JF, Lee S, Nuss M, Park KT, Penz C, Rota J, Schintlmeister A, Schmidt BC, Sohn JC, Solis MA, Tarmann GM, Warren AD, Weller S, Yakovlev RV, Zolotuhin VV, Zwick A (2011) Order Lepidoptera Linnaeus, 1758. In: Zhang ZQ (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148(1): 212–221. https://doi.org/10.11646/zootaxa.3148.1.41

Nieukerken EJ van, Doorenweerd C, Ellis WN, Huisman KJ, Koster JC, Mey W, Muus TST, Schreurs A (2012) *Bucculatrix ainsliella* Murtfeldt, a new North American invader already common on red oaks (*Quercus rubra*) in Western Europe (Bucculatricidae). Nota Lepidopterologica 35(2):135–159.

QGIS (2020) QGIS development team. QGIS geographic information system. Open source geospatial foundation project. https://qgis.org/ja/site/ [date accessed 24.10.2020]

Rambaut A (2018) FigTree v1.4.4. https://github.com/rambaut/figtree [date accessed 15.06.2019]

Seksjaeva SV (1994) New intrageneric groups within the genus *Bucculatrix* (Lepidoptera, Bucculatricidae) distinguished on the basis of the morphology of the male genitalia. Zoologicheskii Zhurnal 73(1): 114–122. [In Russian]

Seksjaeva SV (1998) A comparative analysis of morphological structures of imago as a basis for the classification of mining moths. In: Petersburg S (Ed.) Proceedings of XI Conference of the Russian Entomological Society, St. Petersburg 1, 109–110. [in Russian]

Takeuchi K, Ohbayashi T (2006) A list of moths of the Ogasawara (Bonin) Islands with complete references. Japan Heterocerists' Journal 237: 215–225. [in Japanese]

Tokár Z, Laštůvka A (2018) *Bucculatrix brunnella* sp. n. (Lepidoptera, Bucculatricidae) from Sicily and Sardinia. Nota Lepidopterologica 41(1): 113–117. https://doi.org/10.3897/nl.41.22840

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(1): 232–235. https://doi.org/10.1093/nar/gkw256

Vargas-Ortiz M, Bobadilla D, Huanca-Mamani W, Vargas HA (2018) Genetic divergence of isolated populations of the native micromoth *Bucculatrix mirnae* (Lepidoptera: Bucculatricidae) in the arid environments of Northern Chile. Mitochondrial DNA Part A 29(8): 1139–1147. https://doi.org/10.1080/24701394.2017.1419215

Yonekura K, Kajita T (2003) BG Plants wamei–gakumei index (YList). http://ylist.info [date accessed 05.06.2023]

Supplementary material 1

The list of Bucculatricidae acquired from the BOLD systems for constructing the phylogenetic tree

Authors: Sadahisa Yagi, Johei Oku, Toshiya Hirowatari

Data type: xlsx

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Supplementary material 2

Interspecific and Intraspecific pairwise p-distances in the COI barcode region of the genus *Bucculatrix* similar to *Bucculatrix flavimaculata* sp. nov.

Authors: Sadahisa Yagi, Johei Oku, Toshiya Hirowatari

Data type: xlsx

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